

A novel mechanism for cancer cells to evade immune attack by NK cells

The interaction between NKp44 and proliferating cell nuclear antigen

Benyamin Rosental,¹ Uzi Hadad,¹ Michael Brusilovsky,¹ Kerry S. Campbell² and Angel Porgador^{1,3,*}

¹The Shraga Segal Department of Microbiology and Immunology; Faculty of Health Sciences; Ben-Gurion University of the Negev; Beer Sheva, Israel; ²Institute for Cancer Research; Fox Chase Cancer Center; Philadelphia, PA USA; ³The National Institute for Biotechnology in the Negev; Ben-Gurion University of the Negev; Beer Sheva, Israel

Keywords: NK cells, PCNA, NCR, NKp44, ITIM, inhibitory ligand

Abbreviations: NK, natural killer; PCNA, proliferating cell nuclear antigen; NCRs, natural cytotoxic receptors; ITIM, immunoreceptor tyrosine-based inhibitory motif; NKIS, NK immunological synapse; ANXA2, annexin A2

We recently reported proliferating cell nuclear antigen (PCNA) as a ligand for the NK cell activating receptor, NKp44, which unexpectedly triggers inhibition. The recognition of nuclear proteins such as PCNA, by related NK cell receptors has been reported. Widespread upregulation of PCNA in tumor cells may therefore promote immune evasion.

Introduction

Natural killer (NK) cells provide primary innate immune defense against cancer, and natural cytotoxic receptors (NCRs) are key activating receptors for tumor recognition. The purpose of our recent study was to identify and characterize tumor associated ligands for the NCR, NKp44.¹ It is currently believed that tumors that survive immune surveillance have developed mechanisms to evade the immune system.² Proliferating cell nuclear antigen (PCNA) is commonly overexpressed in cancer cells, where it contributes to cellular proliferation and transformation.³ We recently showed that the interaction of NKp44 with target cell-expressed PCNA paradoxically inhibits NK cell function. We further demonstrated that PCNA promotes tumor survival by suppressing NK activation through the immunoreceptor tyrosine-based inhibitory motif (ITIM) on the NKp44 cytoplasmic domain. Finally, we observed that nuclear/cytoplasmic PCNA from target cells is recruited to the NK

immunological synapse (NKIS) when NKp44 is expressed on the NK cells.

Results

Direct binding of NKp44 to PCNA.

Using recombinant soluble chimeric NK cell receptors fused to the human IgG Fc domain, we showed that PCNA protein manifests binding to NKp44 but not to other NK cell receptors, including LIR1, NKG2D, KIR2DL4, NKp30 and NKp46. Immunoprecipitation studies also confirmed that NKp44 interacts with soluble PCNA and native PCNA from cell lysates, while LIR1 does not. Using surface plasmon resonance analysis, NKp44 displayed a characteristic receptor-ligand binding affinity to PCNA ($K_D = 3.4 \times 10^{-9}$ M).

Target PCNA downregulates NK cell functions. We next expressed PCNA-GFP in HeLa target cells and showed that PCNA overexpression suppressed IFN γ secretion and lysis by NKp44-expressing NK cells. In addition, incubation of NKp44-expressing NK cells (NK92-44)

with blocking anti-NKp44 antibodies abolished the inhibition of lysis.

We further investigated the effect of endogenous PCNA-downregulation using an siRNA approach. Downregulation of PCNA in PANC-1 (pancreas), MCF-7 (breast), DU145 (prostate), HeLa (cervix), and U251 (brain) tumor cells resulted in enhanced lysis by NK92-44 cells. Downregulation of endogenous PCNA in target cancer cells also enhanced IFN γ secretion by NKp44-expressing NK cells, whereas responses by NKp44^{null} NKL cells were unaffected.

PCNA inhibits NK cells function through the ITIM on NKp44. We further tested whether this PCNA-mediated inhibition might involve the ITIM located in the cytoplasmic tail of NKp44. We employed NK-92 cells transduced to express a form of NKp44 in which the cytoplasmic tail is either truncated prior to the ITIM or mutated at the tyrosine site within the ITIM. In this setting, the overexpression of PCNA resulted in significantly reduced functions of NK92 cells expressing wild type, as compared with

*Correspondence to: Angel Porgador; Email: angel@bgu.ac.il
Submitted: 01/12/12; Accepted: 01/13/12
<http://dx.doi.org/10.4161/onci.19366>

the mutated forms of NKp44. Thus, the integrity of the ITIM on the NKp44 cytoplasmic domain is essential for the PCNA-mediated inhibitory effect on NK cells.

PCNA recruitment to the NK immune synapse. We also studied whether soluble PCNA, target cell-conditioned medium, or exosomal fractions could mediate PCNA inhibition of NK cell function. None of these conditions could mimic inhibition of NK cell function by PCNA-expressing target cells. On the contrary, a direct interaction between the NK cell and the target cell was found to be imperative for PCNA-mediated effects, both in vitro and in vivo. Furthermore, using live imaging confocal microscopy, we showed that PCNA in the tumor target cell can be recruited to the NKIS, and that this recruitment correlates with a high surface density of NKp44 on the conjugated NK cell.

Discussion

The overexpression of PCNA by tumor cells strongly correlates with cancer virulence, since PCNA contributes to many survival processes, including DNA replication, DNA repair, and cell-cycle progression.³ Our data suggest that PCNA may additionally promote tumor survival by promoting immune evasion through NKp44-mediated inhibition of NK cell attack. Although PCNA is primarily a nuclear/cytoplasmic factor, other cytoplasmic or nuclear proteins have been shown to serve as ligands to membrane-associated NCRs, notably the NKp30 ligands, CMV-pp65 and nuclear BAT3,^{4,5} and the NKp46 ligand, vimentin.⁶ Collectively, these observations suggest that the localization of NCR ligands need not be primarily restricted to the membrane.

We further hypothesize that the PCNA-NKp44 interaction may also contribute to

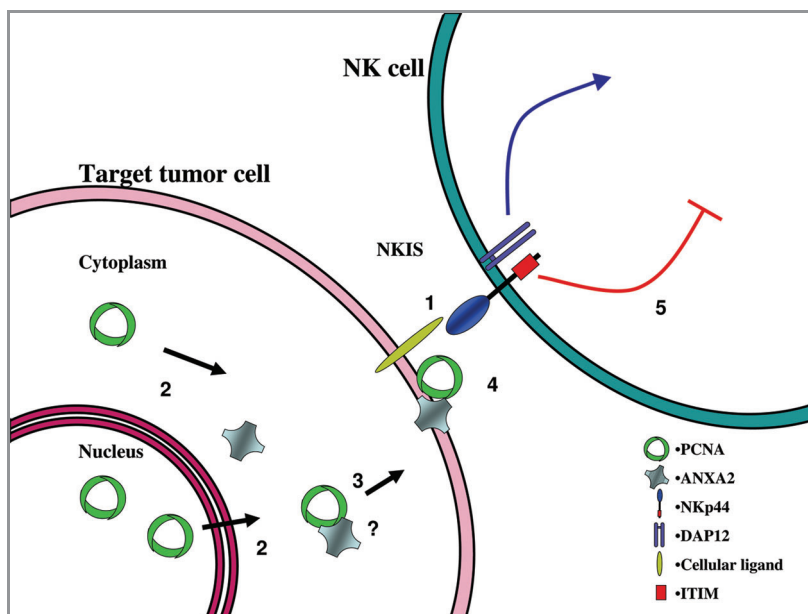


Figure 1. Model of the proposed interaction of NKp44 with PCNA. (1) The interaction with NK cells initiates the movement of PCNA within tumor target cells toward the immunological synapse. PCNA migration within the tumor cell may be initiated by the physical interaction between the NK cell and target cell or the lytic state of other tumor cells attacked by NK cells. (2) Movement of PCNA from the nucleus or the cytoplasm toward the immunological synapse. (3) It is possible that PCNA is carried to or anchored at the membrane by ANXA2. (4) PCNA interacts with NKp44 in the NKIS. (5) The interaction with PCNA uncharacteristically initiates dominant ITIM-mediated inhibitory signaling, which is distinct from the previously-recognized activation signaling of NKp44.

fetal protection from maternal NK cells during pregnancy. Interestingly, PCNA is overexpressed in decidual tissue, and maternal-derived decidual NK cells constitutively express NKp44.⁷ During the first trimester of pregnancy, these decidual NK cells comprise 50% to 90% of the lymphoid cells in the decidua, and they are tolerized toward the fetal tissue.⁷ Interestingly, this NK cell tolerance correlates with PCNA overexpression in fetal-derived trophoblast cells during the first trimester.⁸ Therefore, PCNA-induced inhibition through NKp44 may also contribute to the immune tolerance of maternal NK cells during pregnancy.

Although PCNA has not been previously reported to localize at the plasma membrane, we observed its accumulation

at the contact interface with NKp44-expressing NK cells, which is commonly called the NKIS. PCNA was recently shown to interact with the membrane-associated protein, Annexin A2 (ANXA2),⁹ which was also recently identified as a potential cell surface tumor antigen.¹⁰ Although yet untested, we speculate that ANXA2 may play a role in recruiting PCNA to the NKIS upon interaction of a tumor cell with NKp44-expressing NK cells (Fig. 1).

To summarize, this work identifies a new ligand for NKp44, demonstrates a novel inhibitory signaling function for NKp44, reveals a previously unrecognized mechanism of immune evasion, and re-emphasizes the role of nuclear proteins as important ligands for NCRs.

References

- Rosental B, Brusilovsky M, Hadad U, Oz D, Appel MY, Afegan F, et al. Proliferating cell nuclear antigen is a novel inhibitory ligand for the natural cytotoxicity receptor NKp44. *J Immunol* 2011; 187:5693-702; PMID:22021614; <http://dx.doi.org/10.4049/jimmunol.1102267>
- Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144:646-74; PMID:21376230; <http://dx.doi.org/10.1016/j.cell.2011.02.013>
- Stoimenov I, Helleday T. PCNA on the crossroad of cancer. *Biochem Soc Trans* 2009; 37:605-13; PMID:19442257; <http://dx.doi.org/10.1042/BST0370605>
- Arnon TI, Achdout H, Levi O, Markel G, Saleh N, Katz G, et al. Inhibition of the NKp30 activating receptor by pp65 of human cytomegalovirus. *Nat Immunol* 2005; 6:515-23; PMID:15821739; <http://dx.doi.org/10.1038/ni1190>
- Pogge von Strandmann E, Simhadri VR, von Tresckow B, Sasse S, Reiners KS, Hansen HP, et al. Human leukocyte antigen-B-associated transcript 3 is released from tumor cells and engages the NKp30 receptor on natural killer cells. *Immunity* 2007; 27:965-74; PMID:18055229; <http://dx.doi.org/10.1016/j.immuni.2007.10.010>

6. Garg A, Barnes PF, Porgador A, Roy S, Wu S, Nanda JS, et al. Vimentin expressed on Mycobacterium tuberculosis-infected human monocytes is involved in binding to the NKp46 receptor. *J Immunol* 2006; 177:6192-8; PMID:17056548
7. Vacca P, Cantoni C, Prato C, Fulcheri E, Moretta A, Moretta L, et al. Regulatory role of NKp44, NKp46, DNAM-1 and NKG2D receptors in the interaction between NK cells and trophoblast cells. Evidence for divergent functional profiles of decidual versus peripheral NK cells. *Int Immunol* 2008; 20:1395-405; PMID: 18815119; <http://dx.doi.org/10.1093/intimm/dxn105>
8. Korgun ET, Celik-Ozenci C, Acar N, Cayli S, Desoye G, Demir R. Location of cell cycle regulators cyclin B1, cyclin A, PCNA, Ki67 and cell cycle inhibitors p21, p27 and p57 in human first trimester placenta and deciduas. *Histochem Cell Biol* 2006; 125:615-24; PMID:16491347; <http://dx.doi.org/10.1007/s00418-006-0160-y>
9. Babiychuk EB, Draeger A. Annexins in cell membrane dynamics. Ca(2+)-regulated association of lipid microdomains. *J Cell Biol* 2000; 150:1113-24; PMID: 10973999; <http://dx.doi.org/10.1083/jcb.150.5.1113>
10. Zheng L, Foley K, Huang L, Leubner A, Mo G, Olino K, et al. Tyrosine 23 phosphorylation-dependent cell-surface localization of annexin A2 is required for invasion and metastases of pancreatic cancer. *PLoS One* 2011; 6:e19390; PMID:21572519; <http://dx.doi.org/10.1371/journal.pone.0019390>

© 2012 Landes Bioscience.

Do not distribute.